

## Evaporative water loss and colour change in the Australian desert tree frog *Litoria rubella* (Amphibia: Hylidae)

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**Abstract** – The desert tree frog, *Litoria rubella*, is a small (2–4 g) frog found in northern Australia. These tree frogs typically rest in a water-conserving posture, and are moderately water-proof. Their evaporative water loss when in the water-conserving posture is reduced to  $1.8 \text{ mg min}^{-1}$  ( $39 \text{ mg g}^{-1} \text{ h}^{-1}$ ) and resistance increased to  $7.3 \text{ sec cm}^{-1}$ , compared with tree frogs not in the water-conserving posture ( $7.6 \text{ mg min}^{-1}$ ,  $173 \text{ mg g}^{-1} \text{ h}^{-1}$ ,  $1.1 \text{ sec cm}^{-1}$ ). When in the water-conserving posture and exposed to dry air, the tree frogs dramatically change colour from the typical gray, brown or fawn, to a bright white. The toe-web melanophore index decreases from 3.8 for moist frogs, to 2.3 for desiccated frogs. The high skin resistance to evaporation and white colour of tree frogs when exposed to desiccating conditions appear to be important adaptations to reduce evaporative water loss and prevent overheating when basking in direct sunlight.

### INTRODUCTION

Many species of Australian tree frogs of the genus *Litoria*, are arboreal and frequently perch in exposed sites on vegetation. The desert tree frog, *Litoria rubella* (Gray 1842) is a small, robust species found throughout northern Australia (Cogger 1992; Tyler *et al.* 1994). It survives in arid areas, generally by sheltering in cracks between rocks, or other habitats that provide protection from wind and solar insolation, such as bore holes and water tanks. However, these tree frogs have been observed to perch in sites exposed to direct solar insolation (A.R. Main, *pers. comm.*).

Perching in locations exposed to wind and solar insolation would potentially subject a small frog, such as *Litoria rubella*, to severe desiccation and high body temperatures. Some of the arboreal Australian *Litoria* species have been reported to have reduced rates of cutaneous evaporative water loss (Withers *et al.* 1984; Buttemer 1990) and this would minimise the potential for desiccation when basking. The objective of this study was initially to examine the rate of cutaneous evaporative water loss for the desert tree frog, but preliminary observations revealed that these tree frogs often blanched in colour to a bright white when exposed to dry air. Desert tree frogs are generally grey, brown or fawn in colour, with a broad dark vertebral band and a black stripe along the side of the head (Cogger 1992; Tyler *et al.* 1994). Consequently, the study was extended to investigate the potential roles of ambient humidity and background colour in controlling skin colour.

### MATERIALS AND METHODS

Desert tree frogs were collected from a bore on Mallina Station ( $26^\circ \text{ S}$ ,  $114^\circ \text{ E}$ ), in the arid Pilbara region of Western Australia. They were transported to the laboratory in Perth, for study. Body mass was measured to  $\pm 1 \text{ mg}$ ; mass ranged from 1.6 to 4.3 g.

The rate of evaporative water loss was determined for individual *L. rubella* at an ambient air temperature of  $22\text{--}24^\circ \text{ C}$ , using flow-through hygrometry. Compressed air was dried by passage through a column of silica gel, and flowed at a rate of  $500 \text{ ml min}^{-1}$  through a glass tube containing a frog. The excurrent air was passed through a Western Digital DP1100 dewpoint hygrometer for determination of the water content of the air. The analog voltage output of the hygrometer was monitored at 30 second intervals by a PC, and the dewpoint converted to absolute humidity ( $\text{mg H}_2\text{O L}^{-1}$ ) using the psychrometric equations of Parrish and Putnam (1977). The rate of evaporative water loss (EWL;  $\text{mg H}_2\text{O min}^{-1}$ ) of the frog was calculated from the absolute humidity of the incurrent and excurrent air, and the mass specific evaporative water loss (MSEWL;  $\text{mg H}_2\text{O g}^{-1} \text{ h}^{-1}$ ) was then calculated from the body weight (measured to  $\pm 0.001 \text{ g}$ ). The exposed dorsal surface area of the frog (whether it was or was not in the water-conserving posture) was estimated to be  $2/3$  of the total body surface area (see Withers *et al.* 1982a; Buttemer 1990) calculated from body weight by the allometric equation of McClanahan and Baldwin (1969) *i.e.*  $\text{cm}^2 = 2/3 \cdot 9.9 \text{ g}^{0.567}$ . The

surface-area specific evaporative water loss was calculated and converted to resistance by assuming that the gradient in water vapour density was the difference between the vapour density for air saturated at the ambient temperature and the vapour density of excurrent air. The resistance for frogs not in the water-conserving posture was not recalculated although their exposed surface area would have been higher than that of frogs in the water-conserving posture; this means that their resistance was potentially underestimated by as much as 33% (if all of the skin were exposed). The calculated resistance includes some respiratory water loss, hence the actual cutaneous resistance is slightly lower than the calculated value. No correction was made for the air boundary layer to resistance.

For behavioural observations, eight frogs were placed individually in small plastic containers, either white or black in colour, with a transparent lid. The frogs were transferred to the glass tube, with either humid air or dry air flow, and kept in darkness for about 1 hour. The frogs were then removed from the container, and their colour was noted, and eight chromatophores on the transparent toe webbing of a hind foot were quantified by examination under a dissecting binocular microscope for their chromatophore index (CI; Hogben and Slome 1931).

A dark-background and a light-background adapted frog were sacrificed, and their dorsal skin removed and placed in a diffuse reflectance accessory of a Varian dual-beam spectrophotometer (DMS-80), and the reflectance of the skin determined for wavelengths from 200 to 900 nm.



Figure 1 Light-adapted (right; chromatophore index = 2) and dark-adapted (left; chromatophore index = 4) desert tree frogs, *Litoria rubella*.

## RESULTS

The rate of evaporative water loss of desert tree frogs was relatively high, about  $6 \text{ mg min}^{-1}$  or  $120 \text{ mg g}^{-1} \text{ h}^{-1}$ , if they did not adopt a water-conserving posture in the hygrometer (Table 1), or were placed on a mesh platform to prevent them from concealing their ventral surface from the dry air stream. The skin resistance to evaporative water loss was about  $1.1 \text{ sec cm}^{-1}$  for these frogs, which is similar to the resistance of a free water surface (about  $1 \text{ sec cm}^{-1}$ ). In contrast, tree frogs which adopted a water-conserving posture and concealed their ventral surface from the dry air stream, had a considerably lower evaporative water loss ( $1.2 \text{ mg min}^{-1}$ ,  $26 \text{ mg g}^{-1} \text{ h}^{-1}$ ) and a higher resistance of  $7.3 \text{ sec cm}^{-1}$ .

The desert tree frogs were observed to change colour dramatically, varying from bright white to a dark brown/black (Figure 1). The chromatophore index varies from 1 (fully aggregated) to 5 (fully dispersed; see Figure 2), and the index of toe-web melanophores was generally found to reflect the dorsal colour of the frog. The index changed according to the general body colour, from 2 (almost completely aggregated for pale frogs) to 5 (fully dispersed for dark frogs). For frogs kept on a white background, the CI was  $2.5 \pm 0.3$  ( $n=9$ ), which was significantly different ( $t_{15} = 4.7$ ,  $P < 0.0002$ ) from the CI of frogs on a dark background of  $3.9 \pm 0.2$  ( $n=9$ ). For frogs from black or white backgrounds and maintained in the dark, in moist or dry air, the chromatophore index (Table 2) was significantly related (2-factor ANOVA) to whether the air was moist or dry ( $P < 0.0005$ ) but not to the original background colour ( $P > 0.80$ ). Frogs in dry air had lower melanophore indices than frogs in moist air *i.e.*

Table 1 Body mass and rates of evaporative water loss and resistance for the desert tree frog *Litoria rubella*, when not in, and in, the water-conserving posture. Values are mean  $\pm$  standard error, with the number of observations (n).

	Not in Water Conserving Posture (n=8)	In Water Conserving Posture (n=11)
Body mass (g)	$3.12 \pm 0.23$	$2.64 \pm 0.26^{\text{NS}}$
EWL ( $\text{mg min}^{-1}$ )	$7.63 \pm 1.91$	$1.84 \pm 0.14^*$
MSEWL ( $\text{mg g}^{-1} \text{ h}^{-1}$ )	$173.2 \pm 31.6$	$38.8 \pm 5.9^*$
Resistance ( $\text{sec cm}^{-1}$ )	$1.09 \pm 0.13$	$7.29 \pm 0.58^*$

\* Significant difference between posture groups ( $P < 0.05$ ), by Student t-test.

<sup>NS</sup> No significant difference between posture groups ( $P > 0.05$ ), by Student t-test.

**Table 2** Index for chromatophores in the transparent toe webbing of the desert tree frog *Litoria rubella*, kept in moist or dry air after transfer from black or white backgrounds. Values are mean  $\pm$  standard error;  $n = 8$  for all groups.

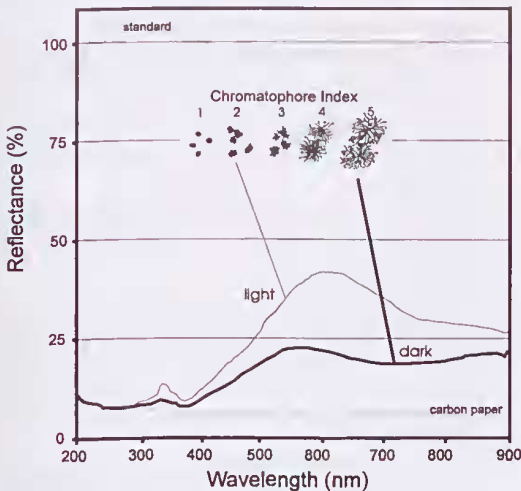
	DARK <sup>2</sup>	LIGHT <sup>2</sup>
MOIST <sup>1</sup>	3.6 $\pm$ 0.4	3.8 $\pm$ 0.4
DRY <sup>1</sup>	2.6 $\pm$ 0.2	2.3 $\pm$ 0.1

<sup>1</sup> significant difference ( $P < 0.0005$ ) by two-factor ANOVA

<sup>2</sup> no significant difference ( $P > 0.8$ ) by two-factor ANOVA

their melanophores were more aggregated and the frogs were lighter in colour.

The reflectance of the dorsal skin from a dark-coloured tree frog (toe web chromatophore index = 5) varied considerably over the visible spectrum (Figure 2), but was lower than for the dorsal skin of a light-coloured tree frog (toe web chromatophore index = 2). The mean reflectance for the skin samples, from 400 to 700 nm (visible spectrum), was 18.8% for the dorsal skin of the dark tree frog, and 31.8% for the dorsal skin of the light tree frog.



**Figure 2** Reflectance of dorsal skin for a light individual of *Litoria rubella* (toe-web chromatophore index = 2) and a dark individual (chromatophore index = 5).

## DISCUSSION

Tree frogs, being generally arboreal, are potentially able to bask although there are few specific reports of basking behaviour. *Litoria caerulea* has been reported to bask (Brattstrom 1970) as have *Litoria chloris* (Buttemer 1990) and some other hylids (*H. labiatus* and *Hyla cinerea*;

Valdivieso and Tamsitt 1974; Freed 1980; Snyder and Hammerson 1993); other frogs also bask (Tyler *et al.* 1983; see Hutchison and Dupré 1992). Being exposed to solar insolation and wind in conspicuous basking sites would potentially desiccate and overheat frogs, particularly smaller species such as *Litoria rubella*.

Most amphibians, which evaporate water from their skin as if it were a free water surface, would rapidly dehydrate when basking in exposed, arboreal conditions (see Shoemaker *et al.* 1992; Hutchison and Dupré 1992). Many such frogs adopt a water-conserving posture when exposed to desiccating conditions; the ventral skin is firmly adpressed to the substrate, the limbs are held tightly under the body, and the head is lowered to protect the gular region (see Stille 1958; Pough *et al.* 1983). *Litoria rubella* routinely adopts such a water-conserving posture when perched, and during measurement of evaporative water loss.

A number of arboreal, basking frogs have a reduced rate of evaporative water loss. For example, the Southern African rhacophorid tree frog *Chiromantis xerampelina* has a very low rate of EWL (Loveridge 1970), as does the South American tree frog *Phyllomedusa xerampelina* (Shoemaker *et al.* 1972) and Southern African hyperoliid reed frogs (Withers *et al.* 1982a,b). Consequently, it is not surprising to note that the Australian tree frog *Litoria rubella* has a reduced EWL (Table 1), as do some other *Litoria* spp (Withers *et al.* 1984; Buttemer 1990), at least when they are in the water-conserving posture with the ventral skin concealed. However, the resistance of *Litoria* spp is intermediate (10–40 sec cm<sup>-1</sup>) between that of typical anuran amphibians ( $\approx 1$ ) and the “waterproof” frogs ( $\geq 100$ ). When *L. rubella* were not in a water-conserving posture, their skin resistance to evaporative water loss was only about 1.6 sec cm<sup>-1</sup>, which is similar to that expected for “non-waterproof” frogs of this size.

Basking in exposed sites presumably elevates body temperature, which confers thermoregulatory advantages (see Hutchison and Dupré 1992). A number of basking frogs (which have a reduced rate of evaporative water loss) blanch to a pale or white colour when basking, presumably to reduce the absorption of incident solar radiation and prevent overheating). The tree frogs *Chiromantis xerampelina* and *Hyperolius* spp are bright white when basking in sunlight (Stewart 1967; Passmore and Carruthers 1979; Kobelt and Linsenmair 1986; Shoemaker *et al.* 1989). The canyon frog *Hyla arenicolor* blanches when basking at high ambient temperature (Snyder and Hammerson 1993). The tree frog *Hyla cinerea* blanches at high ambient temperature (King *et al.* 1994). *Litoria chloris* basks, after feeding (Buttemer 1990). *L. rubella* also basks in sites exposed to solar insolation, and blanches to

a bright white colour (A.R. Main, pers. comm.). The light skin colour increases cutaneous reflectance and decreases the radiative heat load. For example, dorsal skin reflectance increases from 18.8% for dark *L. rubella* to 31.8% for light individuals (Figure 2); the effective solar reflectance (corrected for the spectral energy distribution of solar radiation; Bakken *et al.* 1978) is similar, at 19.7% for dark frogs and 34.7% for light frogs, because of the similar spectral pattern in both reflectance and solar energy distribution. This change is similar in pattern to that reported for other light-adapted and dark-adapted frogs (Deanin and Steggerda 1948), but is greater in magnitude.

In many amphibians, skin colour is matched to the background colour by dispersion or aggregation of the dermal and epidermal melanophores (Waring 1963). However, reed frogs (*Hyperolius* spp.) blanch to a bright white colour when exposed to dry air (Withers *et al.* 1982b) regardless of their background colour, although the physiological significance of this is not immediately apparent as colour does not directly influence the EWL. Similarly, the desert tree frog *Litoria rubella*, blanches when exposed to dry air, regardless of previous background colour (Table 2). I suggest that this colour change is a response to basking conditions (exposure to dry air) that results in an adaptive increase in reflectance which is an important response to a related basking condition, exposure to incident solar radiation. Colour change in response to humidity does also occur in other frogs, but the effect is minor compared with background matching and temperature effects (Hogben and Slome 1931; Waring 1963).

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